Plasmid digestion

Materials and Equipment

- Alcohol lamp
- Microtube rack
- Micropipette
- Micropipette tips
- Microtubes (2 ml)
- Thermoblock

Reagents

- Nuclease free water
- NEB Buffer 10X
- Plasmid
- Restriction Enzymes

Methodology

Each reaction will be added in a 0.2ml tube (for a 50 µL reaction).
Control 1:

A. Add up to 50 µL nuclease free water (32 µL)
B. 5ul Buffer 10X
C. 500 ug plasmid (12 µL)
D. 1µL Restriction Enzyme 1

Control 2:

A. Add up to 50 µL nuclease free water (32 µL)
B. 5ul Buffer 10X
C. 500 ug plasmid (12 µL)
D. 1µL Restriction Enzyme 2

Negative Control:

A. Plasmid without enzymes

Digestion:

A. Add up to 50 µL nuclease free water (32 µL),
B. 5ul Buffer 10X
C. 500 ug plasmid (12 µL)
D. 1µL Restriction Enzyme 1
E. 1µL Restriction Enzyme 2

1. Mix gently each tube.
2. Place at thermoblock, 37˚C, 1 hour.
3. Inactivate enzymes at 80˚C for 20 minutes.
4. Store digestions at -20˚C.